COMPARATIVE ANATOMY OF THE LEAF-BEARING CACTACEAE, III

FORM AND DISTRIBUTION OF CRYSTALS IN PERESKIA, PERESKIOPSIS AND QUIABENTIA *

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SINCE THE PUBLICATION of Schleiden's classical paper (1845) on the anatomy of Cactaceae, many references, frequently more or less casual or incidental, have been made to the occurrence of crystals in various representatives of the family. The most comprehensive of these papers is that of Lauterbach (1889) who utilized crystals of calcium oxalate in correlation with mucilaginous and other structures in an attempt to differentiate three categories of the Cactaceae.

Inadequate attention in investigations of cacti has been given to ranges of variability of selected diagnostic characters, not only in different taxa, different collections of the same taxon, and different plants of the same clone, but also in different parts of the same plant during successive stages of its growth to maturity. It seems advisable, accordingly, to determine to what extent crystals provide reliable and significant taxonomic evidence in the case of the leaf-bearing genera of the Cactaceae.

CRYSTALS OF LEAVES

Druses of calcium oxalate occur in the leaves of all putative species of *Pereskia*, *Pereskiopsis*, and *Quiabentia* of which I have succeeded in obtaining material (FIGS. 1–12). This is true, regardless of whether the plants were grown in greenhouses, botanical gardens, or in their native habitats in the wild. The druses vary widely in size, form and number, as they likewise do in the size, form and number of their constituent crystals (FIGS. 13, 15, 16). The conspicuous differences in size (FIG. 1 *vs.* 4), in number (FIG. 3 *vs.* 1 and 4), and in form (FIG. 15 *vs.* 16) suggest at first sight that crystals might be of considerable utility in the differentiation of taxa. Unfortunately, this proves to be uncertain and difficult when the ranges of variability in leaves are taken into consideration. Conspicuous differences such as are illustrated in FIGS. 1–12 may occur at times in different leaves of the same taxon, clone or individual plant. For example, the conspicuous differences between FIG. 7 and FIG. 8 occur in two leaves from the same herbarium specimen. It is evident that differences may occur in leaves during successive stages of their maturation, in

* This investigation was supported by a grant from the National Science Foundation. I am indebted to the American Philosophical Society for the loan of a Wild microscope.

leaves of different sizes, in leaves of plants of different ages, and in those of plants grown under differing environmental conditions. Therefore, the task of securing strictly comparable material of different taxa becomes a difficult and extremely laborious one.

Although a majority of the crystals of calcium oxalate in the leaves of these genera occur in the form of druses having radially oriented individual crystals of varying forms and sizes, aberrant types of crystallization are of not infrequent occurrence.

Particularly where the crystals are very abundant (FIG. 3), some of the cells of the mesophyll may contain a single crystal, several independent crystals or aggregations of crystals that are not perfectly oriented radially.

CRYSTALS OF CORTEX AND PITH

Druses of conspicuously stellate (FIGS. 13, 15) or more rotund (FIGS. 14, 16) form occur in the pith and cortex of all three genera of the leaf-bearing Cactaceae. The size, form, and number of the druses is highly variable, as in the case of the leaf. One or more independent crystals of varying size and form may occur at times in some of the cells of the pith and cortex. It is possibly significant taxonomically that there appears to be marked exaggeration of this tendency in the much expanded pith of large basal stems of *Pereskia conzattii* Britt. & Rose (FIG. 17) and in the cortex of large older stems of *Pereskia colombiana* Britt. & Rose and *P. guamacho* Web.

The most constant and reliable difference, and one of considerable phylogenetic and taxonomic significance, is the concentration of rotund forms of druses in the outermost layer of the cortex of *Pereskiopsis* and *Quiabentia*. In the case of *Pereskia sacharosa* Griseb., *P. grandifolia* Haw., *P. bleo* DC., *P. corrugata* Cutak, *P. tampicana* Web., and allied taxa, young stems of varying diameter, before periderm formation, have a thick cuticle (FIG. 25). The cells of the cortex decrease in size toward the epidermis. Although their cell walls increase somewhat in thickness toward the exterior of the cortex, no clearly differentiated collenchymatous layers are formed subtending the epidermis. The druses are diffusely distributed in the cortex (FIG. 25). In comparable stems of *Pereskiopsis* and *Quiabentia* conspicuous layers of subepidermal collenchyma are formed (FIG. 27). The outermost layer of the collenchymatous zone is packed with druses of rotund, rather than conspicuously stellate, form (FIGS. 19, 23, 26). The larger druse-bearing cells tend to bulge the relatively tenuous cuticle and slender epidermal cells outward (FIG. 27). Apparently with increasing succulence in *Pereskiopsis* and *Quiabentia* the inner cells of the cortex tend to become thinner walled, whereas the outer ones become thicker walled and collenchymatous. The first cork cambium or phellogen usually arises by periclinal divisions of epidermal cells and the periderm intervenes between the remainder of the epidermis and the outermost layer of crystal-bearing collenchyma. Thus, the latter layer persists after periderm formation has occurred and may be detected for some time in older stems.

In the case of *Pereskia aculeata* Mill., *P. colombiana*, *P. guamacho*, *P. cubensis* Britt. & Rose, *P. portulacifolia* Haw., *P. diaz-romeroana* Cárd., *P. weberiana* K. Schum., *P. conzattii*, *P. autumnalis* (Eichlam) Rose, and *P. nicoyana* Web., periderm formation commonly tends to be precocious and the epidermis and the outer layers of the cortex are rapidly modified. Although the stems of these pereskias do not form such clearly and strongly differentiated collenchymatous layers as occur characteristically in *Pereskiopsis* and *Quiabentia*, they do exhibit at times a tendency for the outer cortical cells to have thicker walls. Particularly in *P. diaz-romeroana* and *P. weberiana* some druses of rotund form may occur in the outermost cells of the cortex. This suggests that in these species there may be a tendency toward structural changes such as become exaggerated and dominant in *Quiabentia* and *Pereskiopsis*.

In view of the occurrence of so-called crystalliferous hypodermal layers in other cacti, the phenomenon merits detailed consideration in subsequent discussion of salient trends of phylogenetic specialization in the Cactaceae as a whole.

CRYSTALS OF THE SECONDARY PHLOEM AND XYLEM

Druses of varying degrees of abundance occur in the secondary phloem of the three leaf-bearing genera. Although usually of spherical form, they may at times exhibit curious deviations in elongated parenchymatous elements (FIG. 21). In such cells the druses may have a markedly elongated form and have conspicuously larger crystals at their upper and lower extremities (FIG. 18).

As in the case of the leaf, pith and cortex, cells having one or more independent crystals are of sporadic occurrence. However, it is again noteworthy in this connection that in large stems of *P. colombiana* and *P. guamacho* cells with single crystals may predominate in the older secondary phloem, druses being relatively infrequent in comparison.

Pronounced differences in forms of crystallization of calcium oxalate occur in the rays of the secondary xylem. Where the rays are composed of cells having thick, strongly lignified walls, as in many pereskias, crystals either are absent or, when present, occur in a cell singly (FIG. 22) or as several independent crystals, druses being absent. On the contrary, where the rays or parts of them are composed of thin, unlignified cells, a varying number of the thin-walled cells form druses (FIG. 20). It is significant in this connection from a phylogenetic point of view that changes in the rays and forms of crystallization within them appear to be associated in some way with tendencies toward increasing succulence of stems and roots This is another phenomenon which deserves detailed consideration in subsequent discussion of salient trends of phylogenetic specialization in the Cactaceae as a whole.

SPHEROCRYSTALS AND STRIATED ISOTROPIC BODIES

Möbius (1885) early recorded the occurrence of spherocrystals of calcium oxalate in Cactaceae. His illustrations of the structural features of these crystalline bodies are reproduced by Kohl (1889), and two of them by Solereder (1899) and by Metcalfe and Chalk (1950).

A few finely striated bodies that are birefringent in polarized light occur sporadically in some of my collections of leaf-bearing cacti that are preserved in formalin-acetic-alcohol. Where they occur in small cells, and in one case in the lumen of vessels close to the cambium, they tend to be fan-shaped or hemispherical in surface view, rather than spherical as in large-celled tissue (FIG. 29). Where a cluster of cells contains them, the striations of each body are radially oriented toward the center of the aggregation of cells. In the case of the larger spherical forms, not only are they radially striated, but also at times they exhibit concentric structure as well, thus resembling certain of the spherocrystals illustrated by Möbius. They also show at least superficial resemblances to the finely striated central parts of some of the more rotund forms of druses (FIG. 16).

Furthermore, particularly in the case of the crystalliferous subepidermal layer of *Pereskiopsis*, the druses not only exhibit a radially striated central part (FIG. 23) but also not infrequently are concentrically layered internally (FIG. 28). Such rotund forms of druses differ from the spherocrystals (FIG. 29) in being jacketed by a layer of large, more or less protuberant crystals. This raises a question in need of detailed investigation. Are there transitional forms of crystallization of calcium oxalate between typical stellate druses and structures commonly referred to as spherocrystals? Or are there fundamental differences in chemical composition involved?

In contrast to these relatively infrequently occurring anisotropic bodies are the dark brown, striated, isotropic ones commonly visible during early stages of the clearing of dried leaves in 3 per cent sodium hydroxide at 56° C., i.e., prior to their bleaching and dissolution. Although these bodies vary greatly in size and form, they tend to be more or less conspicuously striated. Some of them exhibit concentricities as well as radial striations (FIG. 30), thus in surface view resembling spherocrystals (FIG. 29). Others are composed of small cells the brown contents of which have striations oriented radially toward the center of the composite mass (FIG. 32). Where the constituent cells of the mass are larger, the radial orientation of the striations is more clearly visible (FIG. 33). In surface view such a mass of isotropic composition bears at least a superficial resemblance to Möbius' figures 10–13 of spherocrystals. Under more prolonged treatment in sodium hydroxide brown structureless globules frequently tend to exude from the striated bodies (FIG. 31), or the whole body may lose its striated structure. Obviously, such bodies are not composed of crystalline calcium oxalate, although they occasionally may contain some in the form of "crystal sand." It should be emphasized in these connections that the brown bodies in dried leaves differ from the spherocrystals dealt with by Möbius (1885), Kohl (1889), Lauterbach (1889), and Michaëlis (1896) in being isotropic rather than birefringent in polarized light.

It is important to determine not only the chemical and physical constitution of crystalline and isotropic bodies that occur in material of cacti, but also to obtain reliable evidence regarding the occurrence of such bodies in normal living tissue prior to desiccation, preservation in alcohol or formalin-acetic-alcohol and subsequent treatments with varying reagents. At present, there is adequate evidence regarding the common occurrence of druses of calcium oxalate in normal unmodified tissues of various Cactaceae, as well as in the leaf-bearing ones. However, there is evidence which indicates that the druses are not composed solely of calcium oxalate, but contain organic substances as well, particularly in their central parts. For example, when druses are treated with haematoxylin prior to the use of reagents which dissolve constituents such as residues of nuclei, protoplasts, etc., their centers stain very dark (FIGS. 14, 19, 20, 23). Furthermore, if the calcium oxalate is dissolved prior to such treatment, a deeply stained central part of the druse persists. (Compare FIGS. 23 and 24.)

The spherocrystals studied by Möbius are stated to occur, at least in certain cases in living tissue, whereas those observed by Lauterbach and Michaëlis occurred in material preserved in alcohol The formation of dark brown isotropic bodies in the leaves of *Pereskia*, *Pereskiopsis*, and *Quiabentia* appear to be induced by desiccation or other modifying treatments. However, their formation in all three genera is indicative of the presence of similar organic substances and may ultimately prove to be of some taxonomic significance in comparisons between the leaves of Cactaceae and those of other families of the dictotyledons, e.g., those included in the Centrospermae. Thus, their chemical composition and factors involved in their formation merit detailed investigation.

CONCLUSIONS

The ranges of variability in the size, form, and number of druses in the leaves of *Pereskia*, *Pereskiopsis*, and *Quiabentia* are so extensive as to render their use difficult and excessively laborious in differentiating the three genera and the species which occur within them.

The most constant and reliable differences from phylogenetic and taxonomic points of view are those that occur in the outer cortex of young stems prior to periderm formation. *Pereskiopsis* and *Quiabentia*, in contrast to *Pereskia*, have conspicuous collenchymatous layers, the outermost of which is packed with druses of rotund form subtending the epidermis.

Another difference of considerable phylogenetic and physiological interest occurs in the rays of the secondary xylem of both stems and roots. Where the rays are composed throughout of cells with thick, heavily lignified walls, as in less modified forms of woody dicotyledons, deposition of calcium oxalate occurs in the form of single crystals or several independent ones, aggregation into druses being absent or of rare occurrence. On the contrary, where the rays or parts of them are composed of cells with thin unlignified walls, such rays or parts contain more or less numerous druses. The changes in wall thickness, lignification and form of crystallization appear to be correlated in some manner with tendencies toward increasing succulence, as in *Pereskiopsis* and *Quiabentia*.

The changes in the outer cortex of *Pereskiopsis* and *Quiabentia* and in the rays of the leaf-bearing cacti appear to be significant in any subsequent discussion of salient trends of phylogenetic specialization in the Cactaceae as a whole.

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EXPLANATION OF PLATES

PLATE I

FIGS. 1–6. Parts of leaves cleared in 3 per cent sodium hydroxide at 56°C., dehydrated, and mounted in diaphane. Druses photographed in polarized light between crossed nicols at a magnification of 150. 1, *Quiabentia zehntneri* (Britt. & Rose) Britt. & Rose [*Rose*, N.Y. Bot. Gard. 42830]; 2, *Pereskiopsis gatesii* Baxter [Gates Cactus Inc.]; 3, *Pereskiopsis diguetii* Britt. & Rose [*Safford*, U.S. Nat. Herb. 2263439]; 4, *Pereskia bleo* DC. [*Pennell 4759* (GH)]; 5, *Pereskia portulacifolia* [*Jiménez 2578* (US)]; 6, *Pereskia aculeata* [*Duss 3073* (GH)].

PLATE II

FIGS. 7–12. Parts of leaves cleared in 3 per cent sodium hydroxide at 56°C., dehydrated, and mounted in diaphane. Druses photographed in polarized light between crossed nicols at a magnification of 150. 7, *Pereskia* aff. *guamacho* [*H. H. Smith 1886* (GH)]; 8, Another leaf from *Smith 1886*; 9, *Pereskia grandifolia* [*Mexia 4129* (GH)]; 10, *Pereskia grandifolia* [*Moran 7270*]; 11, *Pereskia conzattii* [*Boke*]; 12, *Pereskia nicoyana* [Mo. Bot. Gard.].

PLATE III

FIGS. 13–18. Druses photographed in green light at a magnification of 425. 13, *Pereskia diaz-romeroana* [*Cárdenas*], stellate druse from leaf; 14, *Pereskia tampicana* [*Boke B-17*], druse from cortex of stem; 15, *Pereskia* aff. *guamacho* [*Smith 1886* (GH)], stellate druse from leaf; 16, *Pereskia grandifolia* [*Mexia 4129* (GH)], druse from leaf; 17, *Pereskia conzattii* [*Dressler*], crystals from pith of large basal stem; 18, *Pereskiopsis chapistle* Britt. & Rose [*Boke B-3*], druse from phloem parenchyma.

PLATE IV

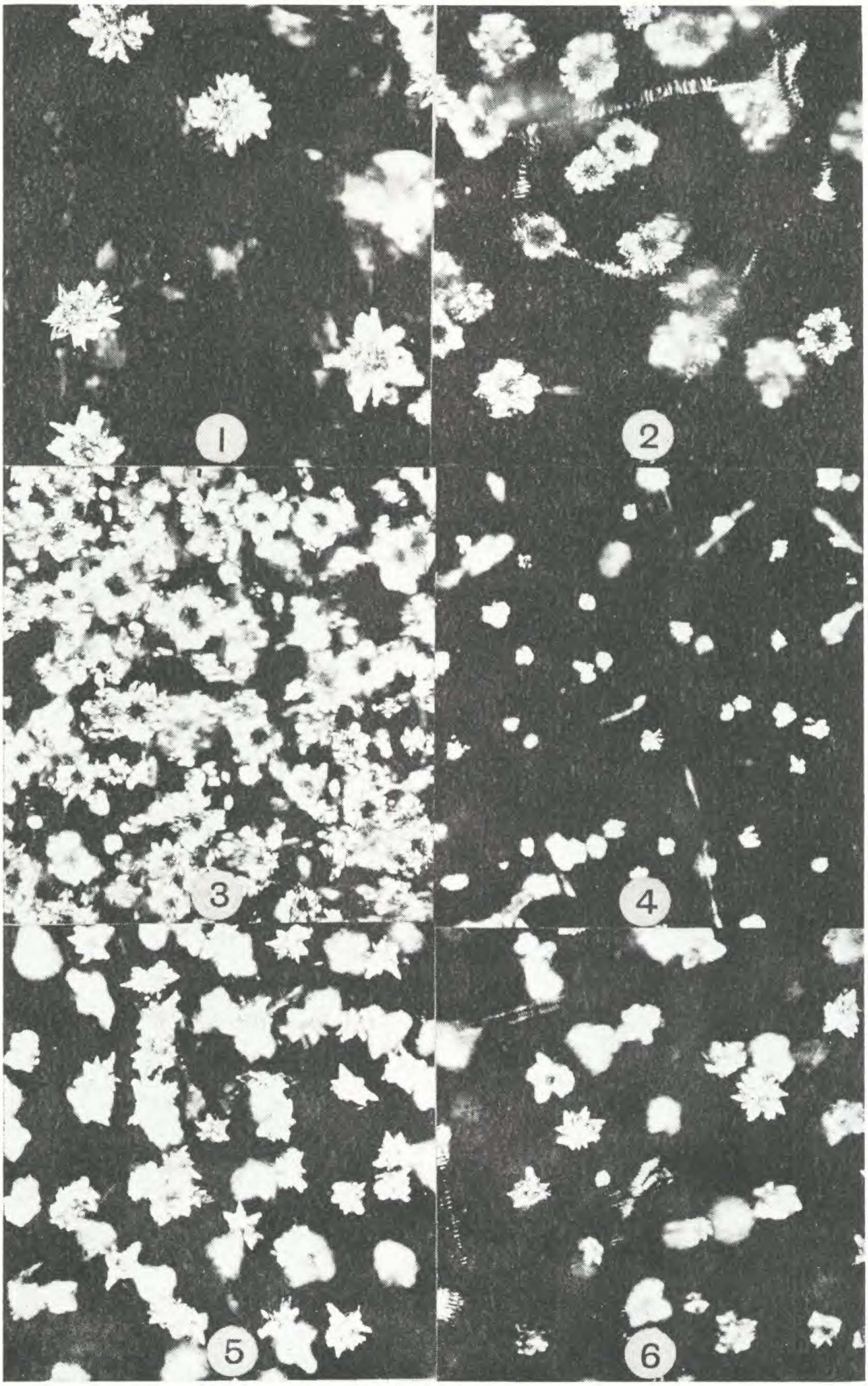
FIGS. 19–24. Varied forms of crystals in stems. 19, *Pereskiopsis chapistle* [Mo. Bot. Gard.], tangential longitudinal section stained in haematoxylin and Sudan III and mounted in glycerin, showing druses in outer layer of collenchyma subtending the epidermis, as seen in polarized light, × 150; 20, *Pereskiopsis chapistle* [*Boke B-3*], tangential longitudinal section of xylem stained in haematoxylin and safranin and mounted in diaphane, showing stellate druses in thin-walled, unlignified ray parenchyma as seen in polarized light, × 150; 21, *Pereskiopsis chapistle* [*Boke B-3*], tangential longtitudinal section of phloem, showing druses in elongated parenchymatous elements, as seen in polarized light, × 150; 22, *Pereskia colombiana* Britt. & Rose [*Romero*], tangential longitudinal section of xylem showing single crystals in thick-walled lignified ray cells, in polarized light, × 150; 23, *Pereskiopsis chapistle* [Mo. Bot. Gard.], single druse from Fig. 19 photographed in green light, × 425; 24, *Pereskiopsis scandens* Britt. & Rose [N.Y. Bot. Gard. 50085], organic residues of such a druse as illustrated in Fig. 23 after removing the crystals of calcium oxalate in a mixture of equal parts of 10 per cent nitric and 10 per cent chromic acids, staining in haematoxylin and safranin and mounting in glycerin, photographed in green light, × 425.

PLATE V

FIGS. 25–27. Transverse sections of stems. 25, *Pereskia sacharosa* [Mo. Bot. Gard.], stained with haematoxylin and safranin, mounted in diaphane, showing thick cuticle, conspicuous epidermis and diffuse distribution of druses in cortex, photographed in green light, × 150; 26, *Pereskiopsis scandens* Britt. & Rose [N.Y. Bot. Gard. 50085], unstained section mounted in diaphane and photographed in polarized light, showing concentration of druses in subepidermal layer of outer collenchyma, × 150; 27, *The same*, section stained in haematoxylin and safranin, mounted in diaphane and photographed in green light, showing collenchymatous layers in contrast to Fig. 25, and tenuous epidermis bulging outward owing to the development of druses shown in Fig. 26, × 150.

PLATE VI

FIGS. 28–33. Crystalline and isotropic bodies in green light; Fig. 28, × 1000; Fig. 29, × 500; Figs. 30–33, × 260. 28, *Pereskiopsis aquosa* (Web.) Britt. & Rose [N.Y. Bot. Gard.], concentrically layered druse; 29, *Pereskia guamacho* [Atkins Gard.], anisotropic "sphaerocrystal" from pith; 30, *Pereskia* aff. *guamacho* [*Smith 1886* (GH)], brown, radially striated, concentrically layered, isotropic mass, foliar; 31, *Pereskia aculeata* [*Ferreira 2316* (GH)], exudation of structureless brown droplets from radially striated, isotropic mass, foliar; 32, *Pereskia grandifolia* [*Moran 7270*], mass of small cells with striated, brown, isotropic contents, foliar; 33, *Pereskia colombiana* [*Record 16495* (GH)], mass of large cells with striated, brown, isotropic contents, foliar.



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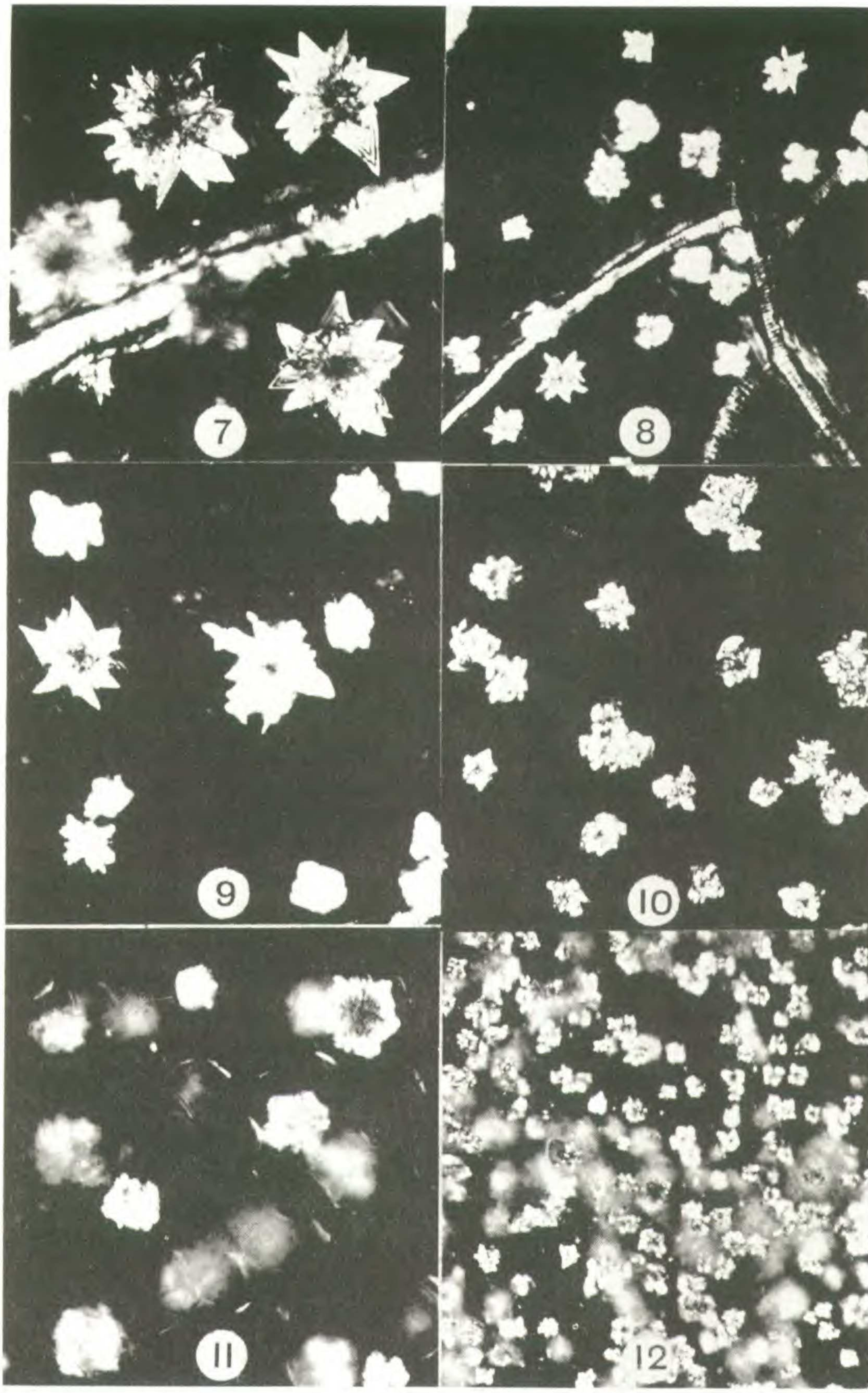

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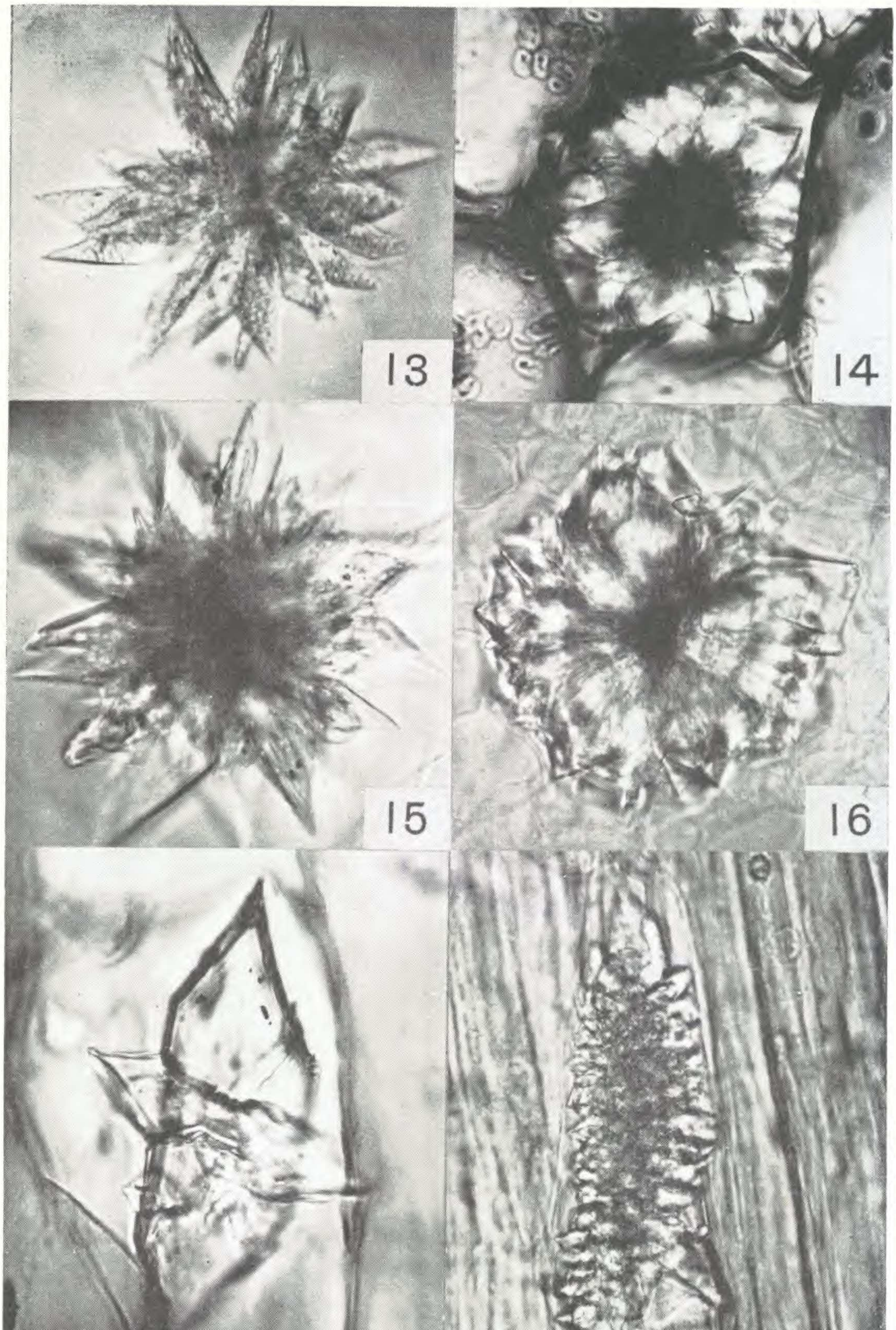

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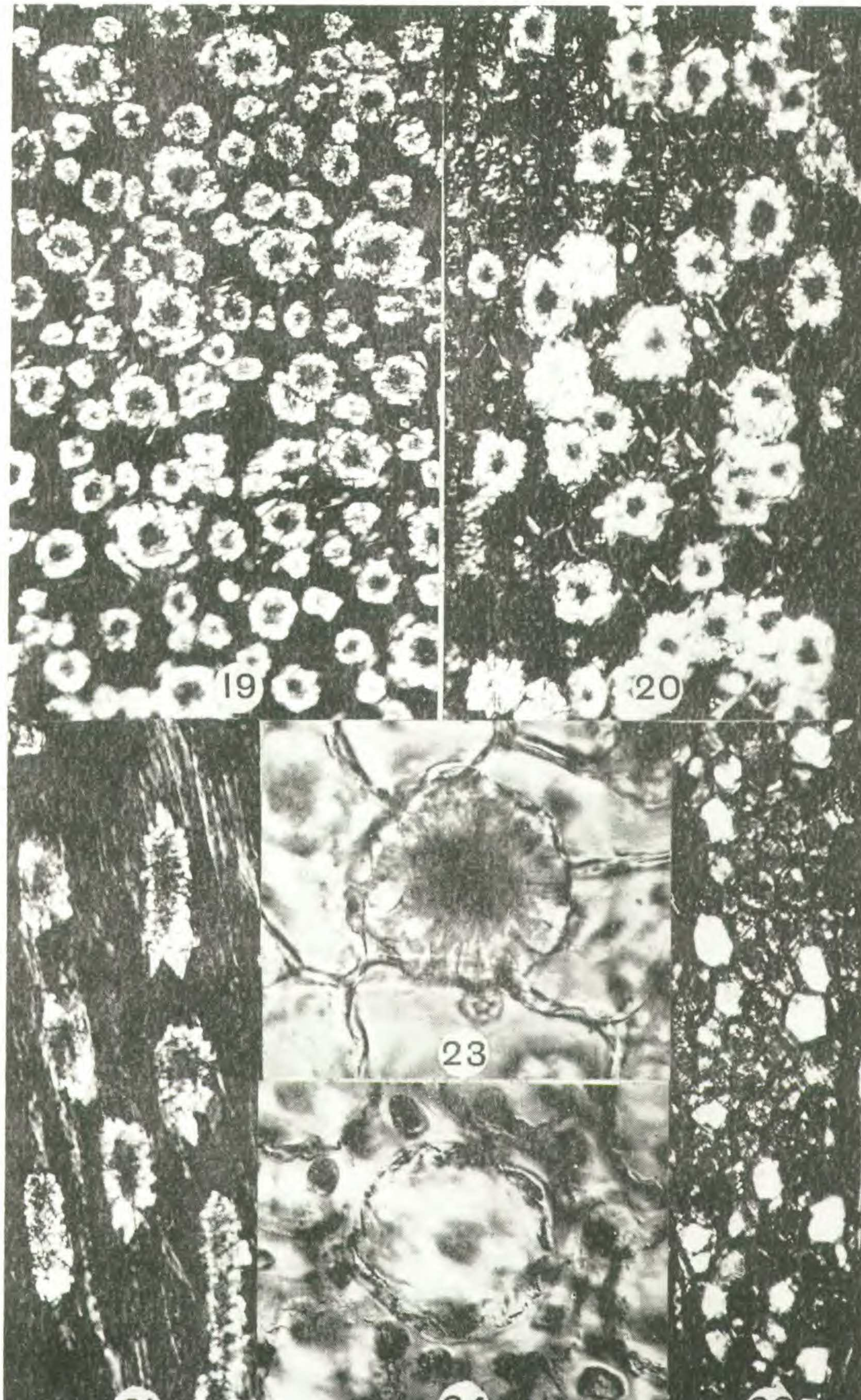

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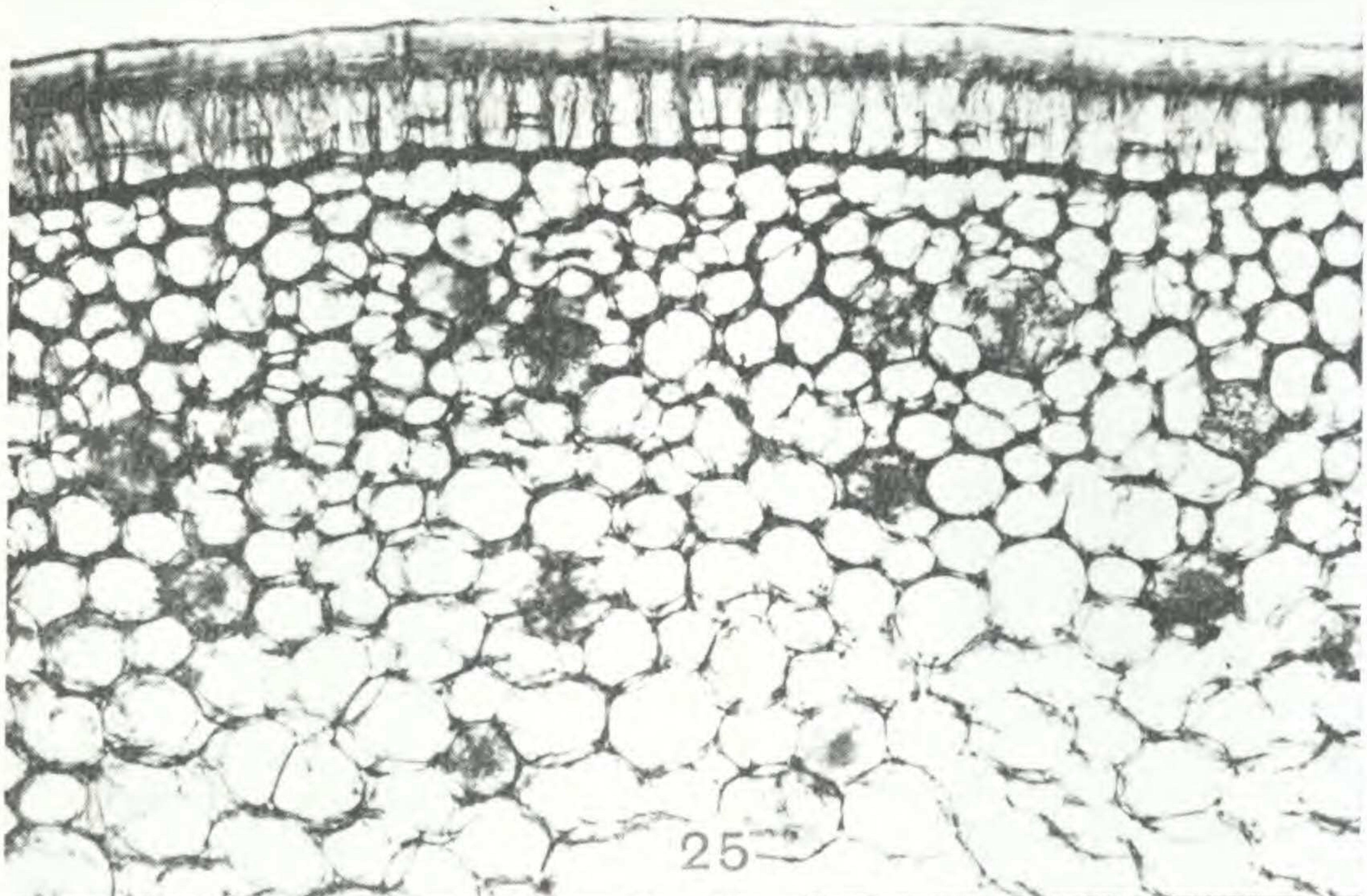

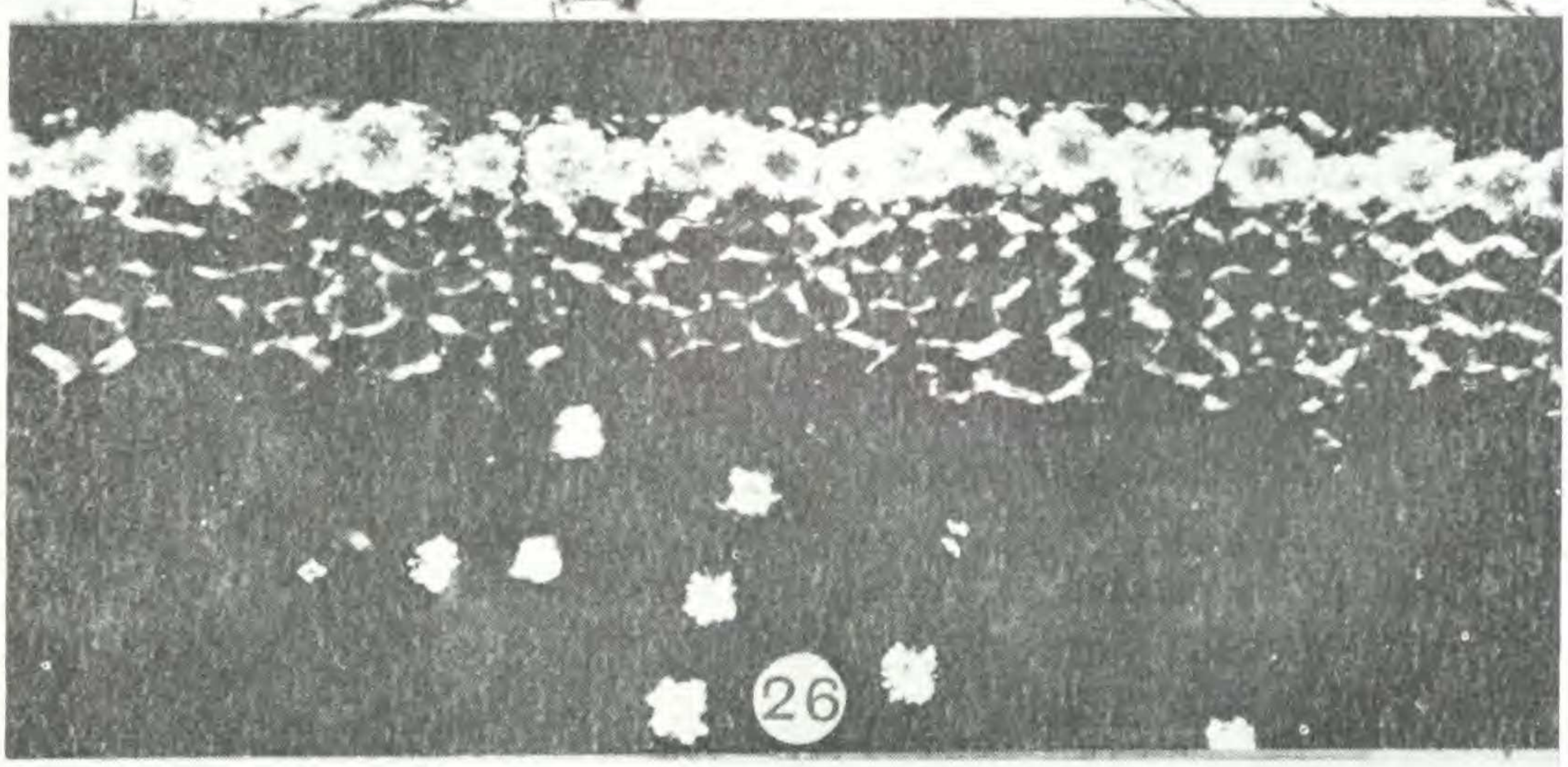

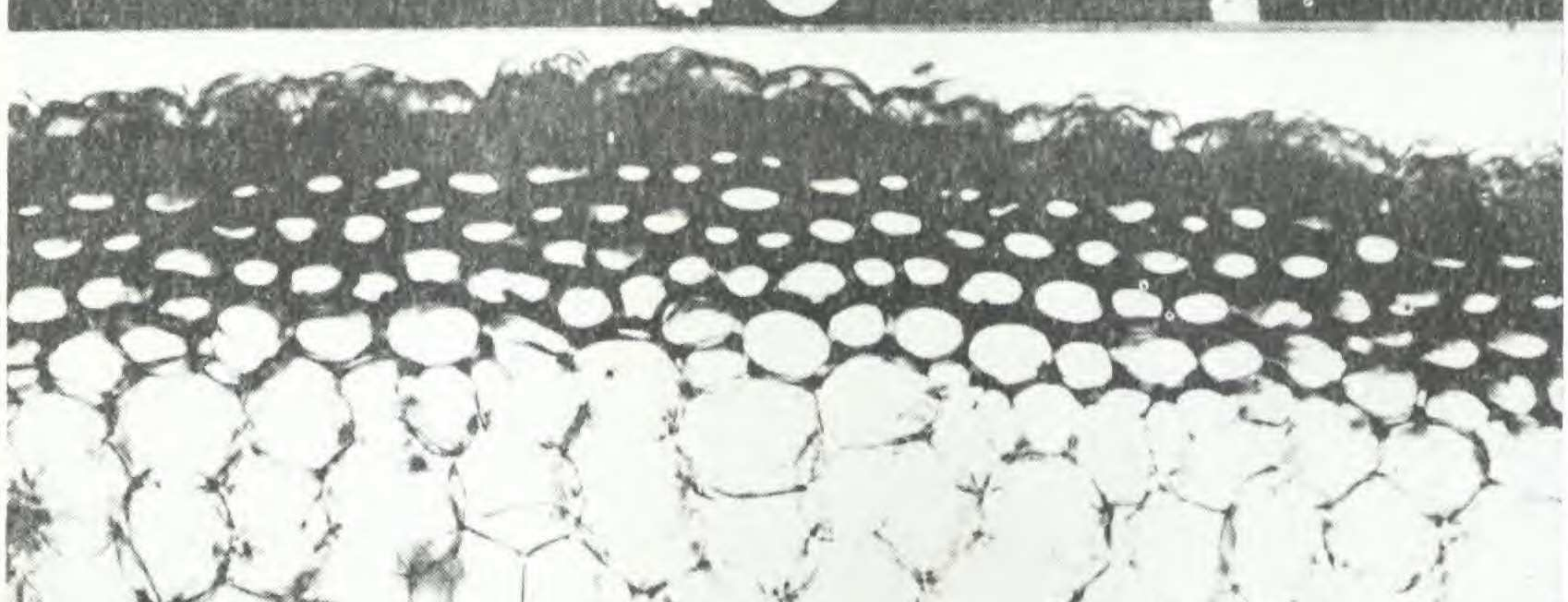

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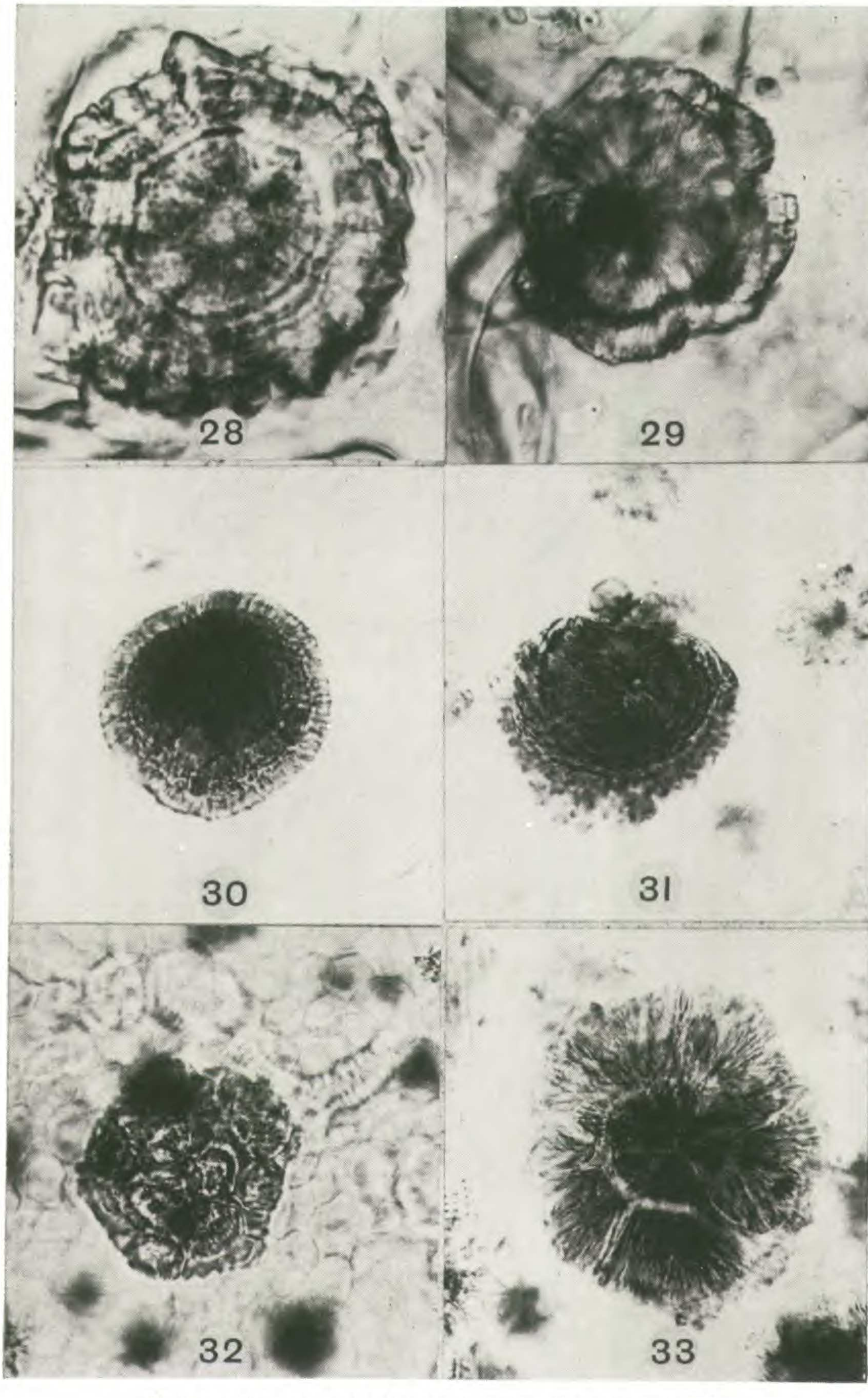

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